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Pressure-dependent changes in the release of GABA by cerebrocortical synaptosomes

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Gilman SC, Colton JS, Dutka AJ. Pressure-dependent changes in the release of GABA by cerebrocortical synaptosomes. Undersea Biomed Res 1989; 16(3):253–258. Previous studies describe a depression in potassium-evoked, calcium-dependent transmitter release from guinea pig cerebrocortical synaptosomes in response to compression to 68 ATA with heliox. The study described in this paper was designed to evaluate whether a similar effect could be detected at lower pressures and to determine whether the effect had an abrupt onset with a fixed response or a progressive response varying over a range of pressures. A typical response to potassium-evoked depolarization was observed at all pressures studied. This consisted of an initial rising phase lasting 2 to 3 min followed by a falling phase. There was a pressure-dependent depression in the absolute amount of transmitter released as well as a depression in the rate of release in the first minute following stimulation. The mean depression in γ-aminobutyric acid release during the first minute was an average of 15% at 19 ATA, 28% at 37 ATA, 38% at 50 ATA, and 54% at 62 ATA when compared to a 1 ATA control.

high pressure nervous syndrome; synaptosome; γ-aminobutyric acid; neurotransmitter; pressure

Exposure of animals to ambient pressures of 19 ATA and greater results in a disturbance of neurologic function referred to as the high pressure nervous syndrome (HPNS). This disorder progresses through a number of symptomatic stages beginning with myoclonic jerks followed by alterations in electroencephalographic (EEG) activity, tremor, and finally convulsions (1–3). Earlier studies have indicated that these clinical manifestations can vary with the particular gas mixture breathed throughout compression, the rate of compression, and the species of animal being compressed. For example, mice subjected to pressurization with He-O_2 demonstrated tremor activity at 75–80 ATA (4), whereas rhesus monkeys convulsed at only 50 ATA (5). Spectral analysis of data from hindlimb movement in guinea pigs revealed that the onset of pressure-induced tremor developed around 19 ATA and increased in severity (i.e., the number of events and amplitude) with compression to 68 ATA (6). Overall, the most predominant clinical signs appear to be neurological.

At the cellular level, neurotransmitter release has been shown to be depressed in various synaptic preparations during exposure to elevated pressures (7–12). For example, intracellular electrophysiologic studies have indicated that He pressure as low as 10 ATA can produce a reduction in the amplitude of the glutamate-mediated excitatory end plate potential in lobster muscle (13). In this same pressure range, miniature end plate potential frequency in the frog neuromuscular junction was reported to be significantly depressed (7). These findings coupled with the data reported by other investigators from studies performed at pressures greater than 10 ATA suggest that the predominant effect of pressure may be on the presynaptic terminal (8, 10–12, 14). It is not surprising then that elevated pressure (68 ATA) could significantly alter calcium-dependent, potassium-evoked [^{3}H]γ-aminobutyric acid (GABA) release from isolated presynaptic terminals (synaptosomes) (15, 16).

The current study was designed to evaluate whether release could be altered (i.e., depressed) at pressures less than 68 ATA. Therefore, in this study, release of [^{3}H]GABA by cerebrocortical synaptosomes was monitored during exposure to a preselected range of helium pressures.

MATERIALS AND METHODS

Animals

Adult male Hartley guinea pigs (300–400 g) were housed at the Laboratory Animal Facility, Naval Medical Research Institute, under a 12-h dark cycle with food and water provided ad libitum.

Materials

Radioactive γ-[2,3-^{3}H]aminobutyric acid (specific activity 25–49 Ci/nmol) was purchased from New England Nuclear Corporation (Boston, MA). All other chemicals were purchased from Sigma Chemical Company (St. Louis, MO).

Method

Each guinea pig was killed by rapid cervical dislocation. The cortex was dissected and synaptosomes prepared by density gradient centrifugation as previously described (15). The method used for loading radioactive GABA into synaptosomes was the same as the procedure described in an earlier publication (17). The final synaptosome pellet was suspended in 10 vol of a buffer medium consisting of a high sodium, calcium-free solution. The composition was (in m*M*): NaCl, 145; KCl, 5; $MgCl_2$, 3.7; KH_2PO_4, 1.2; HEPES, 20; glucose, 10; amino-oxyacetic acid (AOA) 0.1; GABA, 0.01; pH 7.4. Radiolabeled GABA was added and the mixture incubated at 37°C for 5 min to allow uptake by the tissue.

Following uptake, compression studies were conducted using a previously described procedure (16). A brief description follows. A 1.0-ml aliquot of the radioisotope-loaded synaptosome suspension containing 1–1.5 mg protein was placed on a filter unit positioned on a multiperforated support of a perfusion chamber. The perfusion chamber was then connected to a peristaltic pump. After the filter was washed with

50 ml of buffer medium containing 1.2 m*M* Ca^{2+} to remove unbound radioactivity, the filter unit and superfusion apparatus were placed in a hyperbaric chamber. A line from the filter unit was directed over a scintillation vial inserted in a compartment of a turning carousel. This carousel was positioned on a Plexiglas stand, to which was mounted two stepping pump systems. Pump 1 was programmed to deliver 1 ml of buffer medium containing 1.2 m*M* Ca^{2+}; pump 2 to deliver 5 ml of 55 m*M* K^+ containing "efflux" medium. The composition was (in m*M*): NaCl 95; KCl, 55; $CaCl_2$, 1.2; $MgCl_2$, 2.5; KH_2PO_4, 1.2; HEPES, 20; glucose, 10; AOA, 0.1; pH 7.4. Buffer medium was delivered as a bolus over the filter. The hyperbaric chamber was sealed, flushed with pure oxygen for 1 min, pressurized to 1.3 ATA with pure oxygen, and then compressed to a final pressure of 19, 37, 50, or 62 ATA with helium at a compression rate of 3 atm/min. During compression, the filter was washed continuously with buffer medium containing 1.2 m*M* Ca^{2+}. Upon reaching the predetermined pressure, release studies were initiated by delivering 1 ml of buffer medium from pump 1 over the filter unit and two "wash" samples collected directly into scintillation vials. After 2 min at pressure, 5 ml of a depolarizing, 55 m*M* K^+ "efflux" buffer from pump 2 was delivered over the filter unit. Fractions containing 500 μl each of perfusate were collected directly into scintillation vials every minute.

During the release studies the temperature of the buffer media and perfusion chamber was constantly monitored by microthermistors and maintained at 37° ± 1°C. In all experiments aliquots from the same synaptosome preparation were used for obtaining the 1 ATA and increased pressure GABA-release values. Order of experimental exposures was randomized to eliminate any time effect. The control preparations were treated identically to the experimental groups, except that pressure exposures were sham (1 ATA). Fractional [^{3}H]GABA efflux was expressed as percentage of total radioactivity, where total radioactivity was the sum of all fractional filtrate radioactivity and radioactivity remaining on the filter. Statistical significance was determined by analysis of variance (ANOVA). A $P < 0.05$ was considered significant.

RESULTS

Application of incubation media containing 1.2 m*M* calcium and sufficient KCl (55 m*M*) to produce depolarization of the synaptosomes, induced an increase in release of [^{3}H]GABA from the 1 ATA control group and the 4 groups exposed to elevated pressure. All 5 groups maintained a similar release profile, that is, an initial rising phase that lasted 2 to 3 min, followed by a falling phase during the remainder of the observation period (Fig. 1). However, there was a pressure-dependent depression in the amount and rate of the potassium-evoked GABA release from the synpatosomes during minute 1 of transmitter efflux. Mean GABA release during this first minute was decreased by an average of 15% in the 19 ATA group, 28% in the 37 ATA group, 38% in the 50 ATA group, and 54% in the 62 ATA group when compared to the 1 ATA value. Although there was an increase in the rate of release observed in minute 2, the amount of stimulated GABA efflux at increased pressure remained depressed from the 1 ATA control level (Fig. 1).

The effect of pressure on [^{3}H]GABA release was assessed using a 5 × 7 [pressure dosage (1, 19, 37, 50, or 62 ATA) by time (1, 2, 3, 4, 5, 6, or 7 min)] ANOVA based

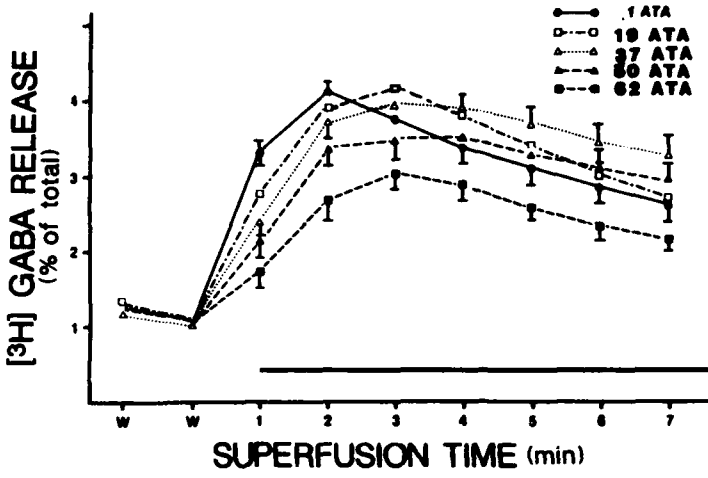

Fig 1. Effect of increased He-O_2 pressure on the depolarization-induced release of [^{3}H]GABA from cerebrocortical synaptosomes. *Each point* represents the mean ± SEM of 12 experiments for each pressure condition.

on repeated measurements. Results revealed a significant pressure effect and time-pressure interaction. As seen in Fig. 1, mean level of GABA release at 1 min was reduced from the 1 ATA control for 37, 50, and 62 ATA. At 2 min the reduction remained only in the 50 and 62 ATA groups.

In addition to evaluating the effect of pressure on the amount of [^{3}H]GABA released during a given time period, the rate of transmitter efflux was also analyzed. The rate of [^{3}H]GABA release is presented for the first 4 min after stimulation with perfusion media containing 55 m*M* K^+ (Table 1). In the first 1 min of observation there was a pressure-dependent decrease in the rate of transmitter efflux, with GABA release slowing from 2.18% per min at 1 ATA to 0.70% per min at 62 ATA (Table 1). In minute 2 of observation, the rates of [^{3}H]GABA release at all elevated pressures were greater than control (1 ATA) values. In addition, there was a slight increase in rate at 50 and 62 ATA from preceding values. In minute 3 the rate of transmitter release at all pressures above 1 ATA dropped below their rates in the first 2 min, and the amount released also peaked at this time (Table 1). In minute 4, rate of release declined for all pressures observed with the exception of 50 ATA (the slight positive release rate at 50 ATA is small and not considered significant) (Table 1).

DISCUSSION

Elevated hydrostatic or helium pressure has been shown to depress the amplitude of intracellularly recorded excitatory and inhibitory junctional potentials from lobster (8–10, 13), reduce peak inward currents recorded in ganglion cells from *Aplysia* or *Helix* (11, 12), and slow the rate of rise of excitatory postsynaptic potentials in voltage-clamped neurons of the squid giant synapse (18). In this study an alteration in the rate and magnitude of transmitter release was observed in guinea pig cerebrocortical synaptosomes in response to helium pressure in the range of 19–62 ATA.

It was within minute 1 of synaptosomal transmitter release that the most profound effects of elevated helium pressure were observed. In this time frame, the depression in the rate and amount of [^{3}H]GABA efflux was progressive and was most predominant at 62 ATA.

TABLE 1
EFFECT OF PRESSURE ON RATE OF STIMULATED [^{3}H]GABA RELEASE BY SYNAPTOSOMES[a]

Pressure ATA	Rate of [^{3}H]GABA Release, %/min			
	Minute 1	Minute 2	Minute 3	Minute 4
1	2.18	0.80	−0.32	−0.40
19	1.57	1.21	0.16	−0.16
37	1.32	1.32	0.20	−0.03
50	1.09	1.21	0.10	0.13
62	0.70	0.93	0.33	−0.13

[a]Values given represent the percent of [^{3}H]GABA released per min and were obtained by determining the difference in percent release at 1-min intervals. For minute 1 values, percent released was subtracted from the percent released in the previous unstimulated wash. Data at each pressure represent the mean of 12 observations at each time period.

The phenomenon of a "progressive" decrease in response has been observed in other systems. For example, Parmentier et al. (12) reported an exponential fall in synaptic currents recorded from *Aplysia*, in the pressure range of 1–100 ATA. Campenot (8) reported a progressive decrease in the excitatory junctional potential amplitude in lobster, Ashford et al. (7) demonstrated an exponential decrease in the frequency of the miniature end plate current at the frog neuromuscular junction, and Grossman and Kendig (10) have recently shown that excitatory junctional potential amplitude in two different species of lobster (*Homarus* and *Panulirus*) are progressively depressed with increasing pressure. The mechanism of the pressure-induced depression is not completely understood at this time, but possible causes may include the following: decreased calcium ion influx through channels in the terminal membrane, interruption of the intraterminal calcium-dependent cascade necessary for transmitter release (e.g., calcium-calmodulin interactions or calcium-dependent protein phosphorylation), vesicle-terminal membrane fusion, level of terminal transmembrane potential, or the rate of transmitter synthesis or replenishment (i.e., re-uptake). Pressure seems to have multiple effects on the terminal, inasmuch as high pressure has been shown to depress calcium ion flux into the terminal (17) and to interfere with membrane fusion (19).

If this depression in transmitter release from potassium-stimulated synaptosomes is representative of release from nerve terminals in intact brain, then depression in the initial rate and amount of transmitter release could significantly alter the timing of the inhibitory and excitatory transmitter interactions. This discrepancy in neuronal synchronization could in turn result in dramatic psychologic and sensory-motor dysfunction such as those that comprise the HPNS.

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The experiments reported herein were conducted according to the principles set forth in the current edition of the Guide for the Care and Use of Laboratory Animals, Institute of Laboratory Animal Resources, National Research Council.

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